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Role of Carbohydrate in Biological Functions of Friend Murine Leukemia Virus gp71

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Purified gp71 of Friend murine leukemia virus (FLV) can interfere with virus infection, absorb neutralizing antibody, and, in the presence of group-specific anti-gp71 antibody, hemagglutinate sheep erythrocytes. Interference by FLV gp71 with several murine leukemia viruses (MuLV) was tested in the XC and S^+L^- assay systems. Treatment of gp71 with trypsin or Pronase eliminated its interfering capacity. However, treatment with neuraminidase or a mixture of glycosidase enzymes, which left the major serological properties of gp71 intact, did not reduce the interference potential of gp71 for FLV or AKR MuLV. The capacity of gp71 to absorb type- or group-specific virus-neutralizing antibodies was similarly affected by the various enzyme treatments. In contrast, indirect hemagglutination by gp71 was abolished not only by proteases but also by treatment with glycosidase enzymes, although neuraminidase had no effect. Preliminary data indicate that infectivity of FLV or xenotropic MuLV was not affected by short treatment with glycosidase enzymes.

Glycoproteins are common components of the envelopes of many viruses which form at the cell surface and have been studied in some detail with regard both to synthesis and biological function in several systems (14, 22-24). However, the role of the carbohydrate moiety in determining the biological properties of viral glycoproteins has not been clearly delineated other than in some agents, such as vesicular stomatitis virus, in which neuraminic acid was found to be required for infection (25). On the other hand, Sindbis virus lacking sialic acid was neither less infectious nor affected in its immunological functions when compared to virus with normal levels of the sugar (26).

The envelope of oncornaviruses plays a major role in virus infectivity, host range, neutralization, interference, and hemagglutination (HA) (for a review, see Schäfer and Bolognesi, Contemp. Top. Immunobiol., in press). The salient component of the viral surface involved in these reactions is a glycoprotein, which in the case of murine leukemia virus (MuLV) has a molecular weight of about 71,000 (gp71) (2, 15). MuLV gp71 possesses multiple antigenic determinants, including type (unique to an individual virus serotype), group (common to viruses from the same species), and interspecies (shared by viruses of other species) (10, 27). Recently the role of carbohydrate in determining several properties of Friend murine leukemia virus (FLV) gp71 was examined (4, 4a). Whereas the digestion of FLV gp71 with several proteases destroyed the polypeptide and eliminated all serological activity, treatment with a mixture of protease-free glycosidase enzymes (termed endoglycosidase) removed about 70% of the carbohydrate without affecting type, group, or interspecies antigenic reactivity as detectable in radioimmunoassays or absorption of cytotoxic antibodies. The present study extends our analysis to several other serobiological properties associated with gp71.

MATERIALS AND METHODS

The isolation and purification of FLV gp71 from the Eveline STU mouse cell line have been detailed elsewhere (15). The antisera to purified gp71 were prepared in goats or rabbits (10). Treatment of gp71 with assorted enzymes was carried out as previously described (4). Briefly, 50 μg of FLV gp71 was incubated with 10 μl of the endoglycosidase preparation (β-galactosidase [0.062 U/ml], β-*N*-acetyglycosaminidase [0.72 U/ml], and endo-β-acetylglucosaminidase from *Diplococcocus pneumoniae*) for 16 h at 37°C in phosphate-buffered saline or with 10 U of highly purified *Vibrio comma* neuraminidase (Behring Werke) for 2 h at 37°C at pH 7.2 or with trypsin (Sigma Chemical Co.) or Pronase (Calbiochem) each at 0.1 mg/ml for 2 h at 37°C at pH 7.2. Enzyme activity was terminated by the addition of an excess of fetal calf serum.

Biological testing of viral interference was per-

formed in the FG-10 clone of sarcoma-positive leukemia-negative (S^+L^-) mouse cells (3). The cells were exposed to an interfering dose (4 μg) of intact or enzyme-treated gp71, mixed with about 100 focus-inducing units of FLV grown in 3T3FL cells (6) or with the AKR MuLV derived from virus-producing AKR+C cells (obtained from R. Friedman via J. Hartley). Alternately, interference kinetic studies with FLV (Fig. 2) were performed in the XC cell assay as described earlier (10). Because no HA was observed with purified gp71 of FLV, indirect HA was performed after the reconstitution of gp71 to multivalent complexes by suitable species-specific antibody (10, 29).

Purified FLV gp71 can induce the formation of type, group, and interspecies neutralizing antibodies in heterologous species (7, 10). To determine the absorption capacity of enzyme-treated and native gp71 for type neutralizing antibodies, a dilution of goat anti-FLV antiserum was used (1:400) that was able to neutralize FLV but not Gross leukemia virus (10). The antiserum was incubated with the respective gp71 samples and the mixture was reacted with FLV. Because residual gp71 that was not bound to antibody could interfere with the FLV test virus, analogous mixtures with normal serum were examined in the interference test by using procedures described previously (10). For absorption of group neutralizing antibodies, an endogenous murine xenotropic virus (MuX) was employed as the test virus. The respective assays for this virus were performed in clone 81 S^+ feline cells as described previously (5, 7).

Standard competition radioimmunoassays with FLV gp71 were performed using procedures outlined in detail elsewhere (4).

RESULTS

We have recently demonstrated that treatment of FLV gp71 with endoglycosidase was effective in removing about 70% of the carbohydrate (4). This was monitored by the loss of [^{3}H]glucosamine label or by the capacity to stain carbohydrate with periodic acid Schiff reagent. Preparations of gp71 used in this study were examined for periodic acid Schiff staining capacity after enzyme treatment and found to behave similarly to those studied previously (4). To insure that the gp71 preparations used (same sample was employed throughout) for the biological studies described below retained their serological potential after enzyme treatment, competition radioimmunoassays were carried out. As shown in Fig. 1, no appreciable loss in the capacity to compete for homologous antibody was observed after treatment with endoglycosidase.

A property of gp71 that conceivably involves carbohydrate is its capacity to bind to sheep erythrocytes (SRBC) as determined by an indirect HA assay (10, 29). As shown in Table 1,

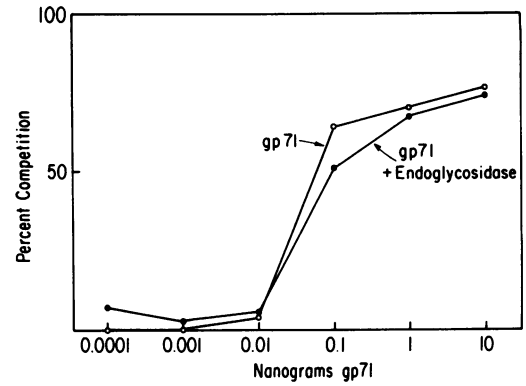

FIG. 1. *Competition radioimmunoassay with FLV gp71 before and after treatment with endoglycosidase. The precipitation of ^{125}I-labeled gp71 (about 2 ng) by goat anti-gp71 antiserum at a limiting dilution (1:2,000) was competed for by increasing amounts of unlabeled native (○) and enzyme-treated (●) gp71 as described earlier (4). Immune complexes were precipitated with rabbit anti-goat IgG, the percentage of ^{125}I counts in the pellets was determined, and the degree of competition was calculated.*

TABLE 1. *Indirect HA with intact or enzyme-treated FLV gp71*

Purified FLV gp71 treated with:	Indirect HA[a]
Control	320 ++++
	640 ++++
Endoglycosidase	5 ++++
	10 +
Neuraminidase	320 ++++
	640 ±
Pronase	<5
Trypsin	<5

[a] The value is the reciprocal of the highest dilution at which HA occurred. The relative strength of the reaction at the limiting dilution is indicated (± to ++++).

native gp71 or gp71 treated with neuraminidase displayed high hemagglutinating activity. Treatment with endoglycosidase, however, essentially eliminated indirect HA activity. Likewise, the ability of whole virus to hemagglutinate SRBC directly was abolished by treatment with endoglycosidase (data not shown). As might be expected, treatment of gp71 with protease enzymes, which completely disrupts the molecule (4), destroyed HA activity. Thus, whereas most serological functions of gp71 are retained after carbohydrate removal (Fig. 1) (4), the capacity to bind to SRBC is lost.

We next examined the interfering capacity of intact or enzyme-treated FLV gp71. The MuLV interference system was originally described

using murine sarcoma virus coated with various types of MuLV in cells preinfected with assorted MuLV (17). An unusual property of the murine system is that, whereas in the avian and feline oncornavirus systems interference is subgroup or type specific (18, 28), several murine viruses cross-interfere although they belong to different serotypes (17). Recently, it was shown that interference could be mediated via the isolated gp71 glycoprotein of FLV (10) with which the same broadly reactive pattern was observed (6, 10).

The capacity of gp71 to interfere with FLV infection, as determined by the XC plaque assay, is shown in Fig. 2. Similar to previous studies (10), low levels (2 ng) of FLV gp71 were sufficient to reduce the plaque number (100 PFU in 0.2 ml of inoculum on 10^6 cells) by about 50%. As shown in Fig. 2, the degree of interference obtained with endoglycosidase-treated gp71 was indistinguishable from that obtained with the native molecule. Additional assays with both FLV and AKR MuLV were performed in mouse S^+L^- cells to determine whether the capacity to interfere with another MuLV was preserved after enzyme treatment (Table 2). As demonstrated earlier (10), intact FLV gp71 could significantly interfere with both serotypes, whereas treatment with trypsin or Pronase totally eliminated this capacity. However, endoglycosidase- or neuraminidase-treated gp71 retained full activity for interference with both FLV and AKR MuLV. Thus, whereas binding to the surface of SRBC requires the integrity of the carbohydrate, the interaction with the cell leading to interference with different MuLV seems to be specified by the protein portion of the molecule.

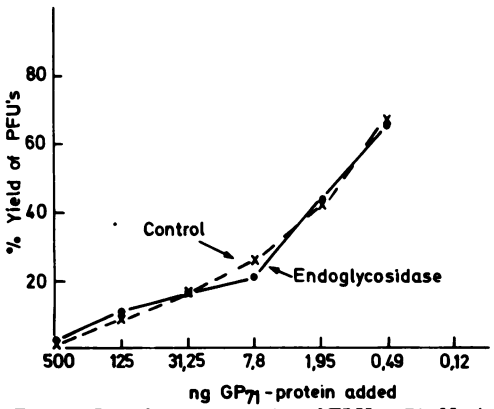

FIG. 2. *Interference capacity of FLV gp71. Native and endoglycosidase-treated gp71 were evaluated in interference tests with FLV in the XC plaque assay (10). The virus inoculum per 10^6 cells was 0.2 ml, containing approximately 100 PFU.*

The capacity of intact and enzymatically treated gp71 to absorb antibodies that neutralize virus was also examined. Goat serum prepared against FLV contains neutralizing antibody in high titer directed against the type and group determinants of the molecule (10). At a dilution of 1:400 this serum is relatively type specific for the neutralization of FLV. Because of the likelihood that excess gp71 (not bound to antibody) would cause interference with FLV in mouse cells, the interfering capacity of the various gp71 fractions used for the absorption of neutralizing antibody was examined concurrently. The results (Fig. 3) are similar to those obtained in previous studies (10) with regard to the capacity of given amounts of gp71 to mediate both processes. As expected, the suppression of plaque formation observed with concentrations of gp71 higher than about 30 ng

TABLE 2. *Interfering capacity of intact or enzyme-treated FLV gp71*

Exposure to and treatment of FLV gp71 (4 μg of gp71/dish)	Avg FIU/dish[a]	
	FLV	AKR
None	135.0	111.0
Untreated	<0.25	0.5
Trypsin treated	137.0	110.5
Pronase treated	108.0	91.5
Neuraminidase treated	0.5	<0.25
Endoglycosidase treated	0.5	<0.25

[a] FIU, Focus-inducing units.

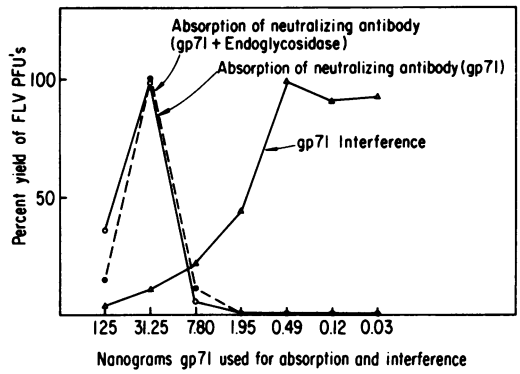

FIG. 3. *Absorption of type-specific neutralizing antibody by FLV gp71. Goat antiserum to FLV reacting type specifically in the neutralization of FLV (dilution, 1:400) was absorbed with various amounts of native gp71 (○) or after treatment with endoglycosidase (●). Details of the procedure have been described earlier (10). Concurrently, similar portions of the respective gp71 preparations were examined for interference capacity (△) as in Fig. 2, except that the appropriate dilutions were made in normal goat serum. A 100% yield is equivalent to 69 FLV PFU.*

(e.g., 125 ng, presumably a level above the binding capacity of the antibody) was due to its interference capacity. However, between 8 and 31 ng of gp71 was able to completely absorb the type-specific neutralizing antibody, with no detectable interference, and this capacity was not affected by treatment with endoglycosidase.

It was also of interest to examine the effect of endoglycosidase treatment on the ability to absorb neutralizing antibodies directed against the group determinant of FLV gp71. MuX assayed in the cat S^+ 81 cells is very sensitive to neutralization by goat anti-gp71, and the reaction can be completely eliminated by preincubation of the antiserum with intact FLV gp71 (7). The heterologous system has the advantage that MuX belongs to a different interference subgroup so that treatment of various cells with FLV gp71 does not result in interference with MuX or its murine sarcoma virus pseudotype (6). Accordingly, if there is an excess of gp71 used for the absorption of antibody, no interference can result in the heterologous system. Table 3 shows that MuX is specifically neutralized by anti-gp71 serum and that preincubation of the antiserum with intact gp71 could remove neutralizing antibody for MuX. The reaction was specific in that another viral envelope surface component, p15(E), was not effective in absorbing this activity although it could absorb the neutralizing activity of anti-p15(E) sera (7). Again, incubation of gp71 with either protease eliminated its ability to react with the antibody directed against the group determinant of MuX gp71. In contrast, treatment with either neuraminidase or endoglycosidase did not diminish the capacity of gp71 to absorb group-specific neutralizing antibody.

Preliminary studies were also carried out to determine the effect on virus infectivity after removal of the carbohydrate moieties from the surface of the virion. Purified Moloney MuLV (M-MuLV) and MuX were each reacted with endoglycosidase, a treatment that was effective in removing similar amounts of carbohydrate from gp71 on the virus surface as with the isolated gp71 (about 70%) (4). A total of 10^6 infectious units each of M-MuLV and MuX derived from tissue culture supernatants were centrifuged, resuspended in 90 μl of phosphate-buffered saline, and reacted with 10 μl of the undiluted glycosidase mixture. The incubation time was shortened to 3 h at 22°C because of the relatively short half-life of virus in phosphate-buffered saline. Glycosidase-treated and control viruses were assayed in their appropriate test systems. Centrifugation and incubation resulted in about 90% loss of virus titer in both control and enzyme-treated M-MuLV or MuX preparations. In neither case could specific loss of infectivity be attributed to endoglycosidase treatment. A similar conclusion could be drawn from preliminary studies with avian oncornaviruses (unpublished data). Further studies are being carried out on the potential inactivation and host range changes of murine oncornaviruses treated with various enzymes.

TABLE 3. *Absorption of group neutralizing antibody from anti-FLV sera by intact or enzyme-treated gp71 from FLV*

Goat anti-FLV gp71 serum (dilution)	Absorbing antigen (8 μg/reaction)	Pretreatment of antigen	MuX surviving[a] fraction (Vn/Vo)
-	-		1.0
-	gp71		0.90
1:100	-		0.003
1:100	gp71		0.91
1:100	gp71	Trypsin	0.003
1:100	gp71	Pronase	0.003
1:100	gp71	Neuraminidase	0.79
1:100	gp71	Endoglycosidase	0.94
1:100	p15(E)[b]		0.005

[a] Significant neutralization is considered to occur when Vn/Vo ≤ 0.1.

[b] Purified from FLV (19).

DISCUSSION

On the basis of previous work (4, 4a), the physicochemical assessment of enzyme-treated gp71 can be summarized as follows: (i) proteases reduce gp71 to smaller polypeptides with the loss of immunochemical reactivity; (ii) endoglycosidase removes about 70% of carbohydrate but leaves antigenic determinants intact; and (iii) neuraminidase removes a small amount of carbohydrate (presumably only neuraminic acid) without affecting the physical and antigenic integrity of the polypeptide. In the present study, three biological functions of isolated gp71 were examined, and all were found to be eliminated by protease treatment. On the other hand, treatment with the endoglycosidase enzyme mixture eliminated the HA capacity but left the interfering and virus-neutralizing antibody absorbing capacities intact. All functions remained after neuraminidase treatment.

With respect to interference, it should be emphasized that, although Gross leukemia virus and FLV are serologically distinguishable, they do cross-interfere (17) and infection with both agents is blocked by FLV gp71 even after endoglycosidase treatment. In contrast, MuX and HIX, the latter a presumptive hybrid of MuX and Moloney MuLV, belong to a distinct inter-

ference group from other ecotropic MuLV's (6). Accordingly, infection of either mouse or cat cells by MuX or HIX is not interfered with by FLV gp71. This was not expected since murine oncornaviruses share at least some gp71 serological determinants on the basis of neutralization (6). Nevertheless, recent studies have indicated that considerable variability can occur in all of the serological specificities of various MuLV gp71's (type, group, and interspecies) (9, 11), whereas those of the internal p30 appear to be more uniform (27). Thus, one has to consider the possibility that only subdeterminants of the group portion of gp71 may be involved in the interference phenomenon.

After removal of most of the carbohydrate, FLV gp71 was also still able to absorb both type and group virus-neutralizing antibodies, as determined in mouse and cat cell assays of FLV and MuX, respectively. Although gp71 type-specific determinants are known to be very important for neutralization, the anti-gp71 hyperimmune serum used in this study contained almost as much group neutralizing antibody (7).

HA was found to be the only function of purified gp71 that was dependent on the presence of carbohydrate. A similar conclusion was reached by Klenk in studies with myxoviruses (12). Treatment with neuraminidase alone, however, did not interfere significantly with the HA activity of FLV gp71, consistent with earlier studies showing that this treatment was actually required to obtain HA activity with whole virus (20, 29). In contrast, in the special case in which mammalian oncornaviruses were used to inhibit HA mediated by myxoviruses, it was found that the presence of oncornavirus-associated neuraminic acid was required (24). Although these studies indicate a major role for the carbohydrate in binding reactions of this nature, it is important to note that protease treatment of gp71 likewise removed HA reactivity. These protease experiments suggest that the organization of the carbohydrate on the native molecule, which probably depends on the protein conformation, is also of critical importance in determining HA activity. However, since the HA measurement requires the presence of antibody, this could possibly reflect the lack of reactivity of the anti-gp71 antibodies with the carbohydrate as suggested by earlier studies (4). These results further indicate that the sites on gp71 which are involved in interference, absorption (and presumably induction) of neutralizing antibody, and possibly infectivity are clearly distinct from those involved in binding to SRBC. This would seem to emphasize the importance of the conformational features of this molecule in influencing its biological properties.

One can speculate on several other functions of carbohydrate chains on glycoprotein molecules that are uniquely important in virion formation as well as in interactions of the virus with the host cell. Many intracellular precursors of structural virus components require proteolytic cleavage, and, in particular, precursors to glycoproteins are glycosylated to some extent prior to cleavage (1, 8, 13; unpublished data). Recent work by Scheid and Choppin (23) points to the necessity for highly specific proteolytic cleavage of paramyxovirus surface glycoproteins for the activation of several biological functions. Recent studies by Nagai et al. (Y. Nagai, H. Ogura, and H.-D. Klenk, submitted for publication) indicated that proper cleavage probably determines virulence of Newcastle disease virus. These studies suggest that host range, tissue specificity, pathogenicity, and, by inference, oncogenicity might be determined by the presence of specific proteases in the target cell and their capacity to mediate the appropriate cleavage of the virion glycoproteins. Certainly the structural positioning of the carbohydrate could play a central role in this process, and the contribution of the host cell in this regard must be considered. This was emphasized by recent work demonstrating that the oligosaccharide portion of the vesicular stomatitis virus glycoprotein varied depending on the cell in which the virus replicated (16).

In the present system we are continuing to examine the contribution of carbohydrates to oncornavirus infectivity, host range, and interaction with the immune system of the host. The results thus far, however, suggest that most of the known functions of the isolated gp71 glycoprotein are specified by the protein portion of the molecule, although it is recognized that the residual carbohydrate not removed by the endoglycosidase enzyme mixture could play some role.

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LITERATURE CITED

1. Arcement, L. J., W. L. Karshin, R. B. Naso, G. Jamjoom, and R. B. Arlinghaus. 1976. Biosynthesis of Rauscher leukemia virus proteins: presence of p30

and envelope p15 sequences in precursor polypeptides. Virology 69:763-774.
2. August, J. T., D. P. Bolognesi, E. Fleissner, R. V. Gilden, and R. C. Nowinski. 1974. A proposed nomenclature for the virion proteins of oncogenic RNA viruses. Virology 60:595-601.
3. Bassin, R. H., M. Tuttle, and P. J. Fischinger. 1971. Rapid cell culture assay technique for murine leukemia viruses. Nature (London) 229:564-566.
4a. Collins, J. J., L. D. Bolio, T. P. Denny, and D. P. Bolognesi. 1977. Interspecies determinants of Friend leukemia virus antigens involved in cytolysis of virus-producing cells. J. Virol. 21:113-118.
4. Bolognesi, D. P., J. J. Collins, J. P. Leis, V. Moennig, W. Schäfer, and P. H. Atkinson. 1975. Role of carbohydrate in determining the immunochemical properties of the major glycoprotein (gp71) of Friend murine leukemia viruses. J. Virol. 16:1453-1463.
5. Fischinger, P. J., C. S. Blevins, and S. Nomura. 1974. Simple, quantitative assay for both xenotropic murine leukemia and ecotropic feline leukemia viruses. J. Virol. 14:177-179.
6. Fischinger, P. J., S. Nomura, and D. P. Bolognesi. 1975. A novel murine oncornavirus with dual eco- and xenotropic properties. Proc. Natl. Acad. Sci. U.S.A. 72:5150-5155.
7. Fischinger, P. J., W. Schäfer, and D. P. Bolognesi. 1976. Neutralization of homologous and heterologous oncornaviruses by antisera against the p15(E) and gp71 polypeptides of Friend murine leukemia virus. Virology 71:169-184.
8. Halpern, M. S., D. P. Bolognesi, and L. J. Lewandowski. 1974. Isolation of the major glycoprotein and a putative precursor from cells transformed by avian sarcoma viruses. Proc. Natl. Acad. Sci. U.S.A. 71:2342-2346.
9. Hino, S., J. R. Stephenson, and S. A. Aaronson. 1976. Radioimmunoassays for the 70,000-molecular-weight glycoproteins of endogenous mouse type C viruses: viral antigen expression in normal mouse tissues and sera. J. Virol. 18:933-941.
10. Hunsmann, G., V. Moennig, L. Pister, E. Seifert, and W. Schäfer. 1974. Properties of mouse leukemia viruses. VIII. The major viral glycoprotein of Friend leukemia virus. Seroimmunological, interfering, and hemagglutinating capacities. Virology 62:307-318.
11. Ihle, J. N., T. P. Denny, and D. P. Bolognesi. 1976. Purification and serological characterization of the major envelope glycoprotein from AKR murine leukemia virus and its reactivity with autogenous immune sera from mice. J. Virol. 17:727-736.
12. Klenk, H. D. 1974. Viral envelopes and their relationship to cellular membranes. Curr. Top. Microbiol. Immunol. 68:29-58.
13. Klenk, H. D., W. Wöllert, R. Rott, and C. Scholtissek. 1974. Association of influenza virus proteins with cytoplasmic fractions. Virology 57:28-41.
14. Lazarowitz, S. G., R. W. Compans, and P. W. Choppin. 1971. Influenza virus structural and nonstructural proteins in infected cells and their plasma membranes. Virology 46:830-843.
15. Moennig, V., H. Frank, G. Hunsmann, I. Schneider, and W. Schäfer. 1974. Properties of mouse leukemia viruses. VII. The major glycoprotein of Friend leukemia virus. Isolation and physicochemical properties. Virology 61:100-111.
16. Moyer, S. A., J. M. Tsang, P. H. Atkinson, and D. F. Summers. 1976. Oligosaccharide moieties of the glycoprotein of vesicular stomatitis virus. J. Virol. 18:167-175.
17. Sarma, P. S., M. P. Cheong, J. W. Hartley, and R. J. Huebner. 1967. A viral interference test for mouse leukemia viruses. Virology 33:180-184.
18. Sarma, P. S., and T. Log. 1973. Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization tests. Virology 54:160-169.
19. Schäfer, W., G. Hunsmann, V. Moennig, F. de Noronha, D. P. Bolognesi, R. W. Green, and G. Huper. 1975. Polypeptides of mammalian oncornaviruses. II. Characterization of a murine leukemia virus polypeptide (p15) bearing interspecies reactivity. Virology 63:48-59.
20. Schäfer, W., J. Lange, P. J. Fischinger, H. Frank, D. P. Bolognesi, and L. Pister. 1972. Properties of mouse leukemia viruses. II. Isolation of viral components. Virology 47:210-228.
21. Schäfer, W., and J. Szántó. 1969. Studies on mouse leukemia viruses. II. Nachweis eines virusspezifischen Hämagglutinins. Z. Naturforsch. Teil B 24:1324-1331.
22. Scheid, A., and P. W. Choppin. 1975. Isolation of paramyxovirus proteins and identification of their biological properties, p. 177-192. *In* B. W. J. Mahy and R. D. Barry (ed.), Negative strand viruses. Academic Press Inc., London.
23. Scheid, A., and P. W. Choppin. 1976. Protease activation mutants of Sendai virus. Activation of biological properties by specific proteases. Virology 69:265-277.
24. Schloemer, R. H., J. Schlom, G. Schochetman, P. Kimball, and R. R. Wagner. 1976. Sialylation of glycoproteins of murine mammary tumor virus, murine leukemia virus, and Mason-Pfizer monkey virus. J. Virol. 18:804-808.
25. Schloemer, R. H., and R. R. Wagner. 1974. Sialoglycoprotein of vesicular stomatitis virus: role of the neuraminic acid in infection. J. Virol. 14:270-281.
26. Stollar, V., D. Stollar, R. Koo, K. A. Harrep, and R. W. Schlesinger. 1976. Sialic acid content of Sindbis virus from vertebrate and mosquito cells. Equivalence of biological and immunological viral properties. Virology 69:104-115.
27. Strand, M., and J. T. August. 1975. Structural proteins of mammalian oncogenesis RNA viruses: multiple antigenic determinants of the major internal protein and envelope glycoprotein. J. Virol. 13:171-180.
28. Vogt. P. K., and R. Ishizaki. 1966. Patterns of viral interference in the avian leukosis and sarcoma complex. Virology 30:368-374.
29. Witter, R., G. Hunsmann, J. Lange, and W. Schäfer. 1973. Properties of mouse leukemia viruses. V. Hemagglutination-inhibition and indirect hemagglutination tests. Virology 54:346-358.